COMMENTARY

Keeping It in the Family: Consanguinity Reveals *P4HTM* as a Novel Syndromic Obesity Gene

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Pediatric obesity is a prevalent disorder that tracks across the life course and is associated with poor health outcomes, including type 2 diabetes (1–3). Common genetic variants explain approximately 20–25% of obesity in the population (4–6); however, each of these common variants typically confer only a small effect on obesity risk. Conversely, severe monogenic (sometimes syndromic) obesity is driven by highly penetrant rare variants. Monogenic obesity syndromes commonly present with significant comorbidities, including intellectual, visual, and hearing impairments. In this issue of *Diabetes*, Saeed et al. (7) identify and characterize rare mutations in the *P4HTM* gene, which is associated with severe monogenic obesity. This is an exciting discovery from consanguineous families of Pakistan highlighting a role for P4HTM in the pathogenesis of obesity and the potential for improved genetic diagnosis and customized treatment strategies.

Deleterious mutations in multiple genes have been identified for monogenic obesity. Indeed, there is a degree of overlap in the genes driving the pathogenesis of monogenic and common polygenic obesity, where specific rare deleterious mutations drive risk for the severe monogenic form while different common genetic variants harbored at the same genes, such as *ADCY3*, *MC4R*, and *LEP*, confer obesity susceptibility within the broader general population (8–11). Such well-characterized mutations within gene members of the hypothalamic leptin-melanocortin pathway and other key molecular processes are now well established as specific genetic risk factors for obesity.

However, there remains an unmet need to characterize additional pathogenic variants and the corresponding effector genes for severe obesity. This in turn will inform more accurate diagnostic approaches and drive new therapeutic development via targeting novel gene products and their related biochemical pathways. Consanguineous families offer a major opportunity to reveal further monogenic obesity loci through principally whole-exome sequencing strategies.

The consanguineous nature of specific Pakistani communities has already been highly beneficial for genetic studies. These cohorts, where marriages among relatives are common, display high consanguinity rates and have already proven to be a rich resource for uncovering human genetic knockouts across a diverse range of phenotypes (12). Indeed, studies in a cohort of individuals with severe obesity in Pakistan (SOPP) have already yielded the *ADCY3* gene as a novel syndromic obesity gene (13). In their article, "Biallelic Mutations in *P4HTM* Cause Syndromic Obesity," Saeed et al. (7) add further understanding to monogenic forms of obesity by reporting via SOPP a new mutation in *P4HTM*, which encodes the prolyl 4-hydroxylase transmembrane protein.

All 456 probands with severe obesity from the SOPP were initially screened for mutations in the well-established *LEP* and *MC4R* genes via Sanger sequencing in order to eliminate those individuals testing positive from further investigation. Those testing negative were further analyzed using exome sequencing or augmented whole-exome sequencing (CoDE-seq). To this end, the authors examined 366 SOPP children with severe obesity, but without a genetic diagnosis, versus 1,003 normal-weight individuals from the Pakistan Risk of Myocardial Infarction Study (PROMIS). This effort sought to identify (likely) pathogenic mutations using the decade-old MiST method (mixed-effects test) combined with a rigorous, stepwise data filtration process. In this approach, rare variants

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within a given gene are combined and analyzed as a cluster to identify genes with a significant variant burden. This specific process identified a single gene, *P4HTM*, in which seven different rare, homozygous mutations deemed likely pathogenic for obesity were found in unrelated male probands from consanguineous families, five of which were derived from SOPP and two from cohorts of Indian and Moroccan origin, respectively.

In a subsequent analysis of data from 200,000 UK Biobank participants, it was noted that only heterozygous null variants for this gene were found, but these turned out to be significantly associated with BMI. Furthermore, the GWAS catalog (www.ebi.ac.uk/gwas) of common variants shows that the *P4HTM* locus is associated with the related traits of waist-to-hip ratio, BMI, and height (14–16). Indeed, this aligns with what is stated above, where different variants in the same gene can be associated with severe monogenic or common population-based obesity.

P4HTM is a member of the hypoxia-inducible factors prolyl 4-hydroxylase family (HIF-P4Hs) and plays a key role in adapting to conditions of low oxygen supply. Interestingly, the hypoxia *EPAS1* locus has been similarly implicated for the orthogonal birth weight trait (17). Mutations in the *P4HTM* gene have been previously associated with HIDEA (hypotonia, hypoventilation, impaired intellectual development, dysautonomia, epilepsy, and eye abnormalities) syndrome (OMIM no. 618493) (18), in which obesity has also been reported (19,20). In line with this, the participants with severe obesity with these mutations displayed a range of other abnormalities, including developmental delay and hypotonia. Indeed, three of them died in early life from respiratory infections.

This encoded enzyme is known to be primarily expressed in the brain. *P4HTM* expression was assessed by the authors in available single-cell data, where they observed relatively ubiquitous expression in all regions of the murine and human hypothalamus. As such, one of the potential mechanisms through which *P4HTM* might confer its obesity risk is via appetite regulation, a process where the hypothalamus plays a central role.

Diving deeper with molecular modeling, the authors collectively observed noticeable domain movements when compared with the wild-type protein. But interestingly, serum levels of key metabolic hormones across five affected patients from the SOPP were within the normal range, although one individual did present with hyperinsulinemia.

The compelling evidence presented in this study for the role of the *P4HTM* locus in the pathogenesis of obesity is strengthened further by the previous associations reported for orthogonal traits. Incorporating this gene into a relevant gene panel would therefore likely enhance genetic diagnosis opportunities for families with affected children. This, in turn, could yield valuable information regarding prognosis and potential additional diagnostic measures, such as conducting polysomnography to detect sleep apnea.

One notable strength of the approach used by the authors is the utilization of a stepwise method, which enhances the reliability and rigor of the findings. Furthermore, the analysis of protein structure and stability represents an additional key aspect of this research, further bolstering the robustness of the *P4HTM* finding.

Overall, this study highlights the power of consanguineous families to characterize new effector genes associated with severe obesity. Such a finding has global implications for understanding the pathogenesis of obesity, given that similar cases were also documented beyond Pakistan, namely, in India and Morocco.

It is important to note that further functional follow-up analyses are warranted to gain a deeper understanding of the precise mechanisms underlying these genetic associations at the *P4HTM* locus. Furthermore, this work should serve as a catalyst for additional investigations into the intriguing role of hypoxia processes in determining body weight.

Taken together, the implications of this discovery, and more generally the process of identifying such rare variants for severe obesity, have significant implications for customized treatment strategies in the future.

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